31. (New) An isolated nucleic acid molecule which comprises a nucleotide sequence encoding a variable region of a non-human TCR α or β peptide wherein said TCR is human HLA-restricted and specific for a tumor-associated antigen.

REMARKS

The present claim amendments are fully supported by the application including the Drawings and claims as filed originally.

For instance, the amendment to claims 6, 7 and 11 find particular support at pg. 6, lines 4-15 (disclosing direct coupling of CD receptor transmembrane and cytoplasmic regions to non-human TCR). Particular constructs are shown in Figure 1 and 3A-B. Page 2 of the specification references a variety of suitable CD receptor regions. Figure 3A-B shows the sequence of a CD8 receptor transmembrane and cytoplasmic region directly coupled to a non-human TCR variable region.

Claims 8, 9, 13 and 14 have been amended to improve clarity.

New claim 22 finds specific support at pg. 7, lines 1-5.

New claim 23 is particularly supported by disclosure at pg. 5, lines 9-10.

New claims 24-28 find support in the claims as originally filed as well as Figures 1 and 3A-B.

New claims 29 and 30 featuring an expression vector find specific support at pg. 4, lines 12-13; and Figure 8. See also Figure 3A-B (featuring various encoded leader sequences).

New claim 31 is the same as unamended claim 6.

No new matter has been added by virtue of the claim amendments or new claims.

Before proceeding, it is believed that a brief overview of the invention would be helpful.

Applicants have discovered how to make highly useful non-human TCRs that are restricted to human HLA molecules. Specifically, the non-human TCRs have been made to respond to tumor associated antigen (TAA) presented in the context of human HLA. Normally, the non-human TCRs would only respond to antigen presented by a non-human cognate molecule, typically a murine MHC. Applicants' non-human TCR is best made in the presence of a functional immune system such as that found in a non-human subject. Importantly, **human HLA restriction of Applicants' non-human TCRs cannot be accomplished *in vitro*** including manipulating TCRs in cell culture. Applicants found that the HLA restricted non-human TCRs of the invention can be made by raising the molecules in a non-human subject that has an immune system modified to produce human HLA. See the Summary of the Invention and pgs. 4-5, for instance.

Turning to the Office Action at pg. 2, the disclosure was object to. The objection has been addressed by this submission.

Claims 6-8, 10-11, and 13-18 stand rejected under 102(a) in view of WO 05/06409 to Mule et al. Although Applicants respectfully disagree with the rejection as formulated, basis for it has been addressed.

As cited, **Mule does not disclose any non-human TCRs that are human HLA restricted**. The TCRs cited by Mule will be restricted to the host of origin unless otherwise specified. In particular, none of the TCR molecules provided on pg. 15 and Example 6 of the PCT application are non-human TCRs that have the characteristic of being human HLA restricted. On this ground alone, there is no anticipation and rejection should be reconsidered and withdrawn.

Applicant respectfully disagrees with the rejection on further grounds.

As cited, Mule discloses chimeric TCRs that feature an immunoglobin antigen binding portion. Mule at pg. 15, lines 14-15. The reference further provides that such chimeric TCRs include a signal transducing region that can be a TCR ζ chain. Mule at pg. 15, lines 28-37. Particular constructs are provided at pg. 24 and 25 of Mule (disclosing binding of the ζ region to the antibody variable chains).

In contrast to the cited Mule reference, the isolated nucleic acid molecule of claim 6 encodes a non-human TCR variable region that is directly coupled to a portion of a CD3, CD8 or CD16 receptor. **Applicants' amended claim 6 does not recite a nucleic acid that encodes an immunoglobin antigen binding portion bound to a TCR ζ region as provided by Mule.**

In view thereof, reconsideration and withdrawal of the rejection are requested.

Claims 6-9, 15-17 and 19 stand rejected as being obvious over Engel et al. (1992) *Science* 256: 1318; and WO 95/06409 to Mule. Although Applicants must disagree with the rejection as formulated, basis for it has been addressed.

As cited, **Engel does not disclose any non-human TCRs that are human HLA restricted**. For instance, Engel discloses that the 2B4 cell line from which TCR α and β chain

was isolated is murine MHC restricted. See entire article including pg. 1318, col. 2, last paragraph, pg. 1320, col. 2, last paragraph and pg. 1319, and Figure 1A. In contrast, the claimed invention is an isolated nucleic acid that encodes a non-human TCR that is HLA restricted (not restricted to murine MHC). On this basis alone the rejection should be withdrawn because the cited molecule is not Applicants' invention.

Applicant respectfully disagrees with the obviousness rejection on further grounds.

As relied on, **Engel does not provide for any non-human TCR that binds a tumor associated antigen (TAA).** In contrast, Applicants' invention encodes a non-human TCR that is specific for a TAA. That specificity is not taught or suggested by Engel as cited by the USPTO. Accordingly, the rejection should be withdrawn because the cited molecule is, again, not Applicants' invention.

Additionally, Engel as relied on does not teach or suggest benefits of having nucleic acid that encodes a non-human TCR that binds TAA and is restricted to human HLA. Such molecules, when expressed, are desirably humanized and can help reduce or avoid unwanted side reactions. Economies of production can also be realized especially when the isolated nucleic acid encodes single chain molecules. Unwanted TCR derivative reactions are also avoided. See Applicants' specification at pg. 5, line 19 to pg. 6, line 3.

Mule as relied on fails to remedy these defects either taken alone or in combination with the cited Engel reference.

In view thereof, there is no grounds for a *prima facie* case. Reconsideration and withdrawal of the §103 rejection are respectfully requested.

Claim 12 stands rejected as being obvious over Mule in view of Reinherz (US Pat. No. 6,416,971). Applicants must respectfully disagree. However basis for the rejection has been addressed by this submission.

In particular, **Reinharz as cited does not teach or suggest any single chain and non-human TCR that is human HLA restricted**. On this basis alone the rejection should be withdrawn.

Further, **the Reinharz patent as relied on does not teach or suggest making any** non-human and human HLA-restricted **TCR in which the single chain is coupled to a portion of any CD3, CD8 or CD16 receptor**.

The benefits of having Applicants' non-human TCR restricted to Human HLA has already been discussed. Such advantages are not taught or suggested by Reinharz as relied on. Further, Applicants found that by coupling a transmembrane and cytoplasmic part of the CD3, CD8, or CD16 receptor to the non-human TCR encoded by the claimed nucleic acid, it was possible to assist in producing stable TCR molecules and avoid competition for dimerization with endogenous TCR.

Additionally, the TCR constructs exemplified by Reinherz generate insoluble TCR molecules that require denaturation and refolding steps to yield functional TCR (see Example I and II, for instance). It is not seen how or why one would be motivated to use the linkers taught by Reinherz in a construct encoding a membrane-associated single-chain TCR-ζ chain fusion if that construct was reportedly insoluble.

Claim 7-9 and 13-14 stand rejected under §112, second paragraph, as being unclear for reciting a ζ region or chain. Action at pgs. 5-6. Applicants respectfully disagree. One working in this particular field would understand what is meant by a ζ region or chain particularly in view of

Applicant's disclosure. In particular, pg. 2 of Applicants' specification references several articles in which specific disclosure of a suitable ζ region or chain is made. Moreover, Applicants' specification has disclosed a particular CD8 ζ region in Figure 3A-B.

However to assist prosecution, the amended claims use the phrase " ζ region" to refer to the CD3 receptor part that includes the transmembrane and cytoplasmic portions of that molecule. See pg. 6, lines 7-8 of Applicants' specification.

New claim 31 is the same as unamended claim 6. Applicants respectfully request allowance of the new claim in view of the remarks above.

Although it is not believed that any further fee is needed to consider this submission, the Office is hereby authorized to charge such fee(s) to our Deposit Account No. **04-1105** if it is deemed necessary.

If the undersigned can be of any assistance in expediting the prosecution of this application, or if there are any questions concerning the above submission, the Examiner is encouraged to call the undersigned collect at the number given below.

Attached to this submission is a marked-up version of the changes made to the specification and claims. The attached page is captioned "version with markings to show changes made".

Respectfully submitted,

Date: 19 Dec 02

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VERSION WITH MARKINGS TO SHOW CHANGES MADE

IN THE SPECIFICATION:

Paragraph at pg. 13, lines 7-10, has been deleted and the following paragraph has been inserted:

A preferred vector for the insertion of the modified sequences, pBJ1Neo with a polylinker insertion site is shown in Figure 8. The host vector, pBJINeo is described in [___,] *Mol. Cell Biol.* (1988) 8: 466; the polylinker is described in [by _____,] *Science* (1990) 249: 677.

IN THE CLAIMS:

Claims 6-9, 11 and 13-14 have been amended as follows.

6. (Amended) An isolated nucleic acid molecule which comprises a nucleotide sequence encoding a variable region of a non-human TCR α or β peptide wherein said TCR is human HLA-restricted and specific for a tumor-associated antigen, the variable region of the non-human TCR α or β peptide being directly coupled to a transmembrane and cytoplasmic region of a CD3, CD8 or CD16 receptor.

7. (Amended) The nucleic acid molecule of claim 6 [which comprises the α or β variable region of the said] wherein the transmembrane and cytoplasmic region is [TCR fused to] the ζ region of CD3 [, CD8 or CD16].

8. (Amended) The nucleic acid molecule of claim 7 wherein said ζ region is that of human CD3 [,CD8 or CD16].

9. (Amended) The nucleic acid molecule of claim 6 wherein said non-human TCR is murine.

11. (Amended) The nucleic acid molecule of claim 10 wherein said single-chain TCR consists of the variable a region fused to variable β region by a flexible linker and said β region is fused to a transmembrane and cytoplasmic region of a CD3, CD8 or CD16 receptor [ζ region].

13. (Amended) The nucleic acid molecule of claim 11 wherein said receptor region is ζ of [chain is that of] CD3 [, CD8 or CD16].

14. (Amended) The nucleic acid molecule of claim 13 wherein the chain is derived from human CD3 [, CD8 or CD16].

The following new claims 22-31 have been added.

22. (New) The isolated nucleic acid molecule of claim 6, wherein the tumor-associated antigen is Her2/neu, ras, p53, tyranase, MART, Gp100, MAGE, BAGE, or MUC-1.

23. (New) The isolated nucleic acid molecule of claim 6, wherein the encoded non-human TCR is restricted to HLA A1, A2, A3 or B7.

24. (New) The isolated nucleic acid molecule of claim 6, wherein the encoded TCR comprises covalently linked in sequence: 1) a non-human TCR α or β peptide; and 2) a transmembrane and cytoplasmic region of a CD3 receptor as shown between nucleotide numbers 927 to 1334 of Figure 3A-B.

25. (New) The isolated nucleic acid molecule of claim 10, wherein the encoded single-chain TCR comprises covalently linked in sequence: 1) a non-human TCR α peptide; 2) a flexible linker; 3) a non-human TCR β peptide; and 4) a transmembrane and cytoplasmic region of a CD3 receptor as shown between nucleotide numbers 927 to 1334 of Figure 3A-B.

26. (New) The isolated nucleic acid molecule of claim 24 or 25 further comprising a CD8 hinge as shown between nucleotide numbers 786 to 914 of Figure 3A-B.

27. (New) The isolated nucleic acid molecule of claim 26, wherein the CD8 hinge is directly coupled between the non-human β peptide and the transmembrane and cytoplasmic region of the CD3 receptor.

28. (New) The isolated nucleic acid of claim 6, wherein the CD3, CD8 or CD16 receptor is human.

29. (New) An expression vector comprising the isolated nucleic acid of claim 6.

30. (New) The expression vector of claim 28 further comprising sequence encoding a leader sequence.

31. (New) An isolated nucleic acid molecule which comprises a nucleotide sequence encoding a variable region of a non-human TCR α or β peptide wherein said TCR is human HLA-restricted and specific for a tumor-associated antigen.